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The influence of age and gender on serum dehydroepiandrosterone sulphate (DHEA-S), IL-6, IL-6 soluble receptor (IL-6 sR) and transforming growth factor beta 1 (TGF-β1) levels in normal healthy blood donors

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SUMMARY

Dysregulation of IL-6 synthesis is thought to play a role in the development of a number of age-related conditions, such as rheumatoid arthritis, osteoporosis, atherosclerosis, Alzheimer's disease and B cell malignancies. Recently it has been suggested that the production of IL-6 is influenced by the adrenal hormone dehydroepiandrosterone (DHEA) and its sulphated derivative DHEA-S. In humans we investigated the relationship between DHEA-S, IL-6, IL-6 sR and TGF-β1 in the serum of normal healthy male and female blood donors. Using immunoassay techniques we found that the serum levels of DHEA-S significantly ($P = 0{\cdot}0001$) decreased with age in both males and females. Furthermore, mean DHEA-S levels in all age groups were significantly ($P = 0{\cdot}0001$) higher in males. Such correlations were not apparent for IL-6 using a standard assay, but a high sensitivity assay revealed that serum IL-6 was significantly ($P = 0{\cdot}0018$) positively correlated with age in males only. In addition, serum levels of DHEA-S were significantly ($P = 0{\cdot}048$) negatively correlated with serum IL-6, again in male subjects only. In contrast, serum IL-6 sR and TGF-β1 levels were not correlated with age in either males or females and were not significantly different between the sexes. However, a significant ($P = 0{\cdot}024$) negative correlation between DHEA-S and IL-6 sR was found in males. These studies clearly highlight the complex nature of the relationship between these molecules in the ageing process in normal healthy blood donors and demonstrate the need to use high sensitivity assays when measuring IL-6 in apparently healthy individuals under the age of 70 years.

Keywords ageing dehydroepiandrosterone sulphate IL-6 IL-6 soluble receptor transforming growth factor-beta

INTRODUCTION

Currently there is much interest in immunosenescence. However, it is only recently that we have begun to understand the cellular and molecular changes that take place in the ageing immune system [1,2]. Of special interest is the dysregulation of cytokines in ageing [3–6]. Ageing appears to result in a reduction of the key Th1 cytokine, IL-2 [7,8] and an increase in the Th2 cytokine, IL-6 [8–13].

For a number of reasons the reported increase in IL-6 has attracted much attention among gerontologists. It has been suggested that dysregulation of IL-6 synthesis may play a role in the development of a variety of age-related conditions such as rheumatoid arthritis [14], osteoporosis [15], atherosclerosis [16], Alzheimer's disease [17,18] and B cell malignancies [19]. Furthermore, it has been shown that the production of IL-6 is influenced by the adrenal hormone dehydroepiandrosterone (DHEA) and its sulphated derivative DHEA-S [20–23]. For example, it has been demonstrated that IL-6 levels were elevated in serum samples obtained from aged mice, and this increase in IL-6 could be reversed by supplementing ageing animals with DHEA-S [20]. Similarly, we have previously reported that DHEA and DHEA-S inhibited the production of IL-6 in unstimulated human spleen cells in culture [21]. The circulating levels of DHEA-S are known to decline with age [24–27]. These observations, coupled with the knowledge that circulating levels of DHEA but not cortisol decline with age [28], have led to the belief that certain age-related immune dysfunctions may be prevented or reversed by oral replacement of DHEA [20,29–34].

Collectively, these observations are very important in relation to our understanding and management of a number of age-related

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conditions. However, the studies of age-related increases in circulating IL-6 levels have given rise to conflicting data [35]. A combination of small sample sizes, poorly selected normal healthy donors, variability in the assays employed and problems arising because the data obtained from males and females have been combined has fuelled this disagreement. In this study we have attempted to address some of the problems by studying the relationship between DHEA-S and IL-6 levels in serum obtained from a much larger population of normal healthy blood donors than previously examined. In addition, we have examined the IL-6 sR and TGF-β1 levels in a proportion of our samples. We chose to measure IL-6 sR to see whether the serum concentrations of this molecule alter with age in light of the recent study which suggests that it serves to reduce the bioavailability of circulating IL-6 [36]. Similarly, TGF-β1 was examined since a recent report suggests that DHEA could induce this anti-inflammatory cytokine by murine macrophages [37]. These studies extend our knowledge of these molecules in the ageing process of normal adults and provide a reference base for future studies in this area.

MATERIALS AND METHODS

Serum samples

Serum samples were obtained from normal healthy blood donors and were provided by the Edinburgh and South East Scotland Region Blood Transfusion Service. All samples passed routine Blood Transfusion Service screening and were aliquoted and stored at −20°C within 48 h of withdrawal and assayed within 4 weeks. Repeated freeze/thawing was avoided. Sera from 412 male donors (age range 17–69 years) and 395 female donors (age range 17–68 years) were used in this study.

Donor health criteria

All donors were classified as healthy according to the strict criteria laid down by the Blood Transfusion Service in the UK [38]. Briefly, this means that all new blood donors have been interviewed and examined by a medical consultant to ensure that they pass a stringent health check. Donors are excluded from donating if they have a history involving a number of medical conditions. These include: cardiovascular disease, central nervous system diseases, gastrointestinal diseases, certain infectious diseases, renal diseases, malignancy and respiratory diseases. They are also excluded if they are waiting to see a doctor or other health care professional, having treatment of any kind, have taken any medication in the last 5 days or have had contact with an infectious disease in the last 4 weeks. In addition, all samples that were used tested negative for hepatitis B surface antigen, antibodies to HIV-1, HIV-2, hepatitis C and *Treponema pallidum* (syphilis).

Immunoassays

Serum DHEA-S and IL-6 levels were measured using chemiluminescent assays on an Immulite automated analyser (Euro/DPC, Gwynedd, UK) according to the manufacturer's instructions [39,40]. The reporting range for the Immulite assays was 0·81–27 μM for the DHEA-S assay and 2–2000 pg/ml for the IL-6 assay. For the sake of statistical analysis, samples which fell below the reporting range of the Immulite IL-6 assay were ascribed a value of 1 pg/ml, i.e. half way between 0 and the lower limit of detection. The intra-assay and interassay coefficients of variation (CV) were found to be 6·2% and 7·4% for the DHEA-S assay and 7·8% and 8·1% for the IL-6 assay. Although both DHEA and its sulphated derivative can be readily assayed, DHEA-S is often measured in preference to DHEA, as the circulating levels of the sulphated derivative are approximately 500 times higher [41] because of its much lower metabolic clearance rate and minimal diurnal variation [42].

Because the IL-6 levels in a large number of samples were found to be below the reporting range of the automated Immulite assay, a number of samples from each decade, chosen at random without reference to their Immulite values, were re-assayed with a high sensitivity IL-6 ELISA (Quantikine HS; R&D Systems, Abingdon, UK). Unfortunately, financial constraints prevented the re-assay of all samples. The sensitivity of this assay was approximately 0·1 pg/ml and the intra-assay and interassay CV were found to be 6·1% and 8·2%. In addition, serum IL-6 sR and TGF-β1 levels were measured in a random selection of samples from each decade in the range 20–59 years using ELISAs (Quantikine; R&D Systems). Again, all age ranges could not be included due to financial restrictions. The sensitivities of these assays were 3·5 pg/ml for the IL-6 sR assay and 7 pg/ml for the TGF-β1 assay. The intra-assay and interassay CV were 2·6% and 4·2% for the IL-6 sR assay and 4·9% and 7·7% for the TGF-β1 assay.

Statistical analysis

All data are given as the mean ± s.d. unless otherwise stated. Correlations between serum concentrations of DHEA-S, IL-6, IL-6 sR, TGF-β1 or age were determined by linear regression analysis. Comparisons between groups were assessed using Student's *t*-test. Statistical analysis was performed using StatView 512+ software (BrainPower, Agoura Hills, CA). $P < 0.05$ was considered to be significant.

RESULTS

Serum DHEA-S levels

Age-related and gender-related differences in the concentrations of DHEA-S in the serum of normal healthy blood donors were found and the results are shown in Table 1 and Fig. 1. Table 1 shows the mean DHEA-S levels in a variety of age groups throughout adulthood. Mean levels of DHEA-S in all age groups were significantly ($P = 0.0001$) higher in males. The DHEA-S concentration peaked at age 17–19 years in females and at age 20–29 years in males. Mean values subsequently steadily declined in both males and females, reaching approximately one third of their maximum concentration by age 60–69 years. As previously shown [24,26,27], serum DHEA-S was found to be negatively correlated with age in both males and females (Fig. 1). This age-related decrease in DHEA-S was found to be highly significant in both males and females ($P = 0.0001$).

Serum IL-6 levels

The concentrations of IL-6 in the sera of normal healthy blood donors, measured using the Immulite assay, are shown in Table 1. The serum concentration of IL-6 in 376 male subjects ranged from non-detectable to 41·4 pg/ml, and that in 359 female subjects ranged from non-detectable to 48·5 pg/ml. It should be noted that approx. 60% of the male subjects and 65% of the female subjects had < 2 pg/ml serum IL-6 using this standard assay. As previously indicated, in order to undertake statistical analysis these samples falling below the detectable range of the assay were ascribed a value of 1 pg/ml. The pooled Immulite data presented in Table 1 suggested an increase in circulating IL-6 up to the 30–39 age group

Table 1. Concentrations of DHEA-S and IL-6 (as measured in the Immulite and R&D assays) in the serum of healthy male and female blood donors in different age groups

	Male			Female		
Age group (years)	DHEA-S (μM)	Immulite IL-6 (pg/ml)	R&D IL-6 (pg/ml)	DHEA-S (μM)	Immulite IL-6 (pg/ml)	R&D IL-6 (pg/ml)
17–19	8·10 ± 2·52 (50)	1·47 ± 1·45 (49)	1·16 ± 0·40 (6)	5·15 ± 2·45 (66)	1·48 ± 1·40 (61)	2·13 ± 1·33 (10)
20–29	9·57 ± 3·43 (75)	2·48 ± 2·70 (67)	1·56 ± 1·00 (12)	4·76 ± 2·66 (73)	3·62 ± 6·59 (67)	1·44 ± 0·64 (11)
30–39	7·56 ± 3·22 (89)	3·25 ± 5·41 (82)	1·23 ± 0·54 (12)	4·49 ± 2·04 (69)	2·47 ± 2·75 (63)	1·77 ± 1·47 (11)
40–49	5·79 ± 2·17 (73)	3·14 ± 5·63 (67)	2·20 ± 2·29 (12)	3·90 ± 1·95 (70)	3·63 ± 6·85 (65)	1·38 ± 0·40 (10)
50–59	4·39 ± 2·21 (70)	2·99 ± 3·97 (63)	1·85 ± 1·04 (12)	2·69 ± 1·62 (61)	2·13 ± 2·43 (54)	1·83 ± 0·78 (12)
60–69	3·54 ± 2·49 (55)	2·49 ± 3·55 (48)	3·39 ± 3·08 (13)	2·06 ± 1·29 (56)	1·63 ± 2·01 (49)	3·54 ± 5·29 (13)

Results are means ± s.d. with the number of subjects in parentheses. Samples with IL-6 levels below the reporting range of the Immulite assay (2 pg/ml) have been ascribed a value of 1 pg/ml for statistical purposes, since this is half way between 0 and the lower limit of detection for this assay.

in males and the 40–49 age group in females, after which the mean values declined in both males and females. Although serum IL-6 appeared to be negatively correlated with age in females, this was not found to be significant ($P = 0{\cdot}441$). No significant difference between the sexes was found for IL-6 using the Immulite assay ($P = 0{\cdot}620$).

Because of the high percentage of samples falling below the detectable range of the Immulite assay, serum IL-6 was also measured in a proportion of the samples using a high sensitivity assay. Initially, 26 samples with IL-6 concentrations ranging from 2·0 to 39·8 pg/ml in the Immulite assay were re-assayed using the high sensitivity assay and the results compared. The Immulite method only gives a value for IL-6 if it is above 2 pg/ml. Therefore, all of the samples which fell below this range could not be compared. However, where samples gave positive results in both methods the assays correlated well ($r = 0{\cdot}95$) and the differences between the two assays were not statistically significant ($P = 0{\cdot}09$). The results presented in Table 1 demonstrate that the values obtained for the Immulite assay were generally higher than those obtained with the high sensitivity assay. This was probably due to the fact that non-detectable Immulite samples were ascribed a value of 1 pg/ml whereas the ELISA could detect IL-6 levels as low as 0·1 pg/ml, and also because of the much larger number of samples measured in the Immulite assay. Age-related and gender-related differences in the concentrations of IL-6 in the sera of normal healthy blood donors, measured using the high sensitivity assay, are shown in Table 1 and Fig. 2. The mean serum concentration of IL-6 in 67 male subjects ranged from 0·52 pg/ml to 11·08 pg/ml and that in 67 female subjects ranged from 0·43 pg/ml to 20·92 pg/ml. Using this assay, serum IL-6 was found to be positively correlated with age in males but not in females (Fig. 2). This age-related increase in IL-6 was found to be highly significant ($P = 0{\cdot}0018$) in males but was not significant in females ($P = 0{\cdot}231$), although examination of the pooled data presented in Table 1 appeared to show an increase in IL-6 using the ELISA method for women in the 60–69 years age group. No significant difference between the sexes was found for IL-6 using the high sensitivity assay ($P = 0{\cdot}839$).

Serum DHEA-S and IL-6 levels

Serum levels of DHEA-S were significantly negatively correlated with serum IL-6 (as assessed in the high sensitivity assay) in male subjects only (Fig. 3) ($P = 0{\cdot}048$ for males and $P = 0{\cdot}955$ for

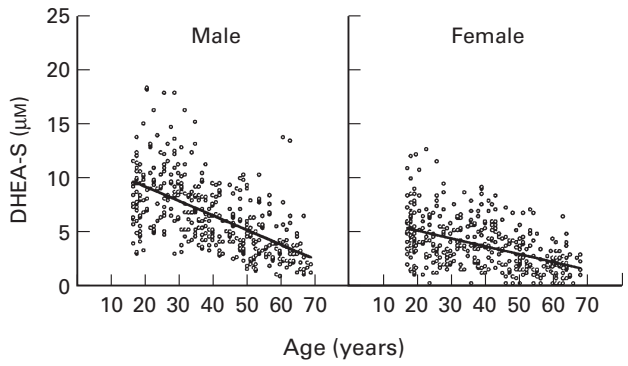

Fig. 1. The correlation between serum DHEA-S levels and age in 412 male and 395 female healthy subjects. The individual values and regression lines are given. Note the highly significant negative correlation of DHEA-S with age in both males ($r = -0{\cdot}582$, $P = 0{\cdot}0001$) and females ($r = -0{\cdot}464$, $P = 0{\cdot}0001$).

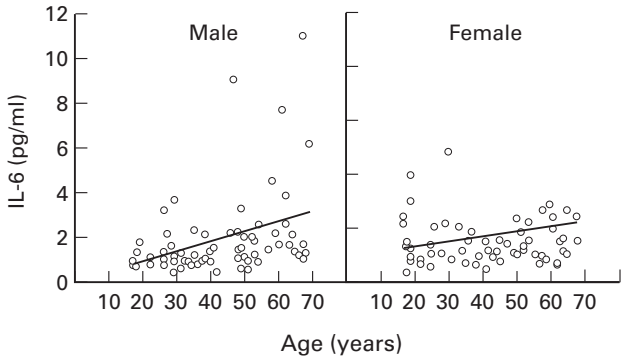

Fig. 2. The correlation between serum IL-6 and age in 67 male and 67 female healthy subjects as revealed using the high sensitivity R&D assay. The individual values and regression lines are given. For clarity a value of 20·92 pg/ml, obtained for one female aged 60 years, has been omitted from the figure but is included in the linear regression and other statistical analyses presented. Note the highly significant positive correlation of IL-6 with age in males ($r = 0{\cdot}375$, $P = 0{\cdot}0018$) but not in females ($r = 0{\cdot}148$, $P = 0{\cdot}231$).

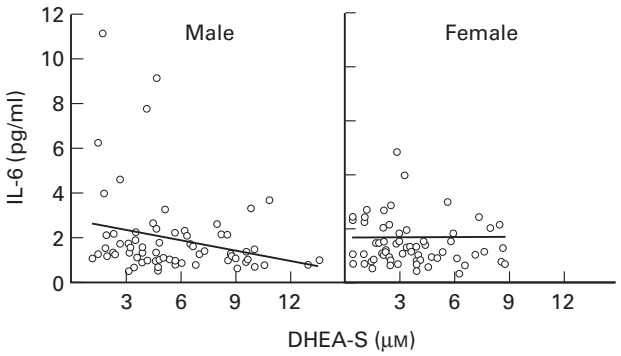

Fig. 3. The correlation between serum DHEA-S and IL-6 (R&D assay) in 67 male and 67 female healthy subjects. The individual values and regression lines are given. Note the significant negative correlation of IL-6 with DHEA-S in males ($r = -0{\cdot}242$, $P = 0{\cdot}048$) but not in females ($r = 0{\cdot}007$, $P = 0{\cdot}955$).

females). Normal healthy male subjects with high DHEA-S serum levels had low IL-6 serum levels and *vice versa*.

Serum IL-6 sR levels

The mean serum concentration of IL-6 sR in 38 male subjects (age range 20–58 years) was $34{\cdot}9 \pm 1{\cdot}28$ ng/ml (ranging from 20·4 ng/ml to 53·1 ng/ml) and that in 38 female subjects (age range 22–58 years) was $33{\cdot}5 \pm 1{\cdot}19$ ng/ml (ranging from 22·5 ng/ml to 50·4 ng/ml). Serum IL-6 sR was not correlated with age in either males or females (Fig. 4) and there were no significant differences between the sexes at all ages ($P = 0{\cdot}408$). However, a negative correlation was found between DHEA-S and IL-6 sR ($r = -0{\cdot}366$) in males but not in females. This DHEA-S-related decrease in IL-6 sR in males was found to be significant ($P = 0{\cdot}024$). In contrast, serum IL-6 sR levels were not correlated with IL-6 levels in either males or females.

Serum TGF-β1 levels

The mean serum concentration of TGF-β1 in 38 male subjects (age range 20–58 years) was $39{\cdot}5 \pm 1{\cdot}30$ ng/ml (ranging from 26·7 ng/ml to 60·8 ng/ml) and that in 38 female subjects (age range 22–58 years) was $38{\cdot}8 \pm 0{\cdot}94$ ng/ml (ranging from 28·4 ng/ml to 53·3 ng/ml). Serum TGF-β1 was not correlated with age in either males or females (Fig. 4), and there were no significant differences between the sexes at all ages ($P = 0{\cdot}376$). In addition, serum TGF-β1 levels were not correlated with DHEA-S or IL-6 levels in either males or females.

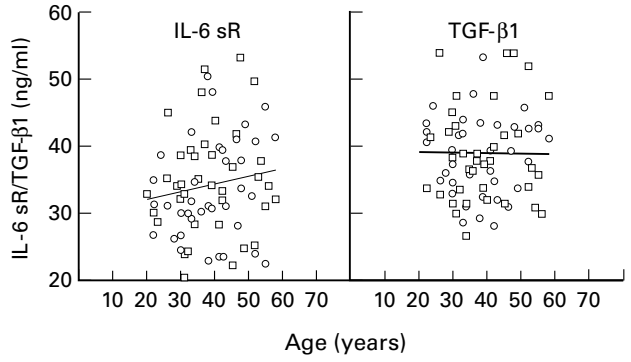

Fig. 4. The correlation between serum IL-6 sR and age and TGF-β1 and age in 38 male and 38 female healthy subjects. The individual values (□, males; ○, females) and regression lines are given. Note that IL-6 sR was not significantly correlated with age ($r = 0{\cdot}155$, $P = 0{\cdot}182$) and neither was TGF-β1 ($r = 0{\cdot}010$, $P = 0{\cdot}931$).

DISCUSSION

In this study we investigated the relationship between DHEA-S, IL-6, IL-6 sR and TGF-β1 in the sera of normal healthy male and female blood donors. While the results for DHEA-S confirm and extend previous data, the relationship between age and IL-6 levels in individuals under the age of 70 years is less clear cut. Furthermore, we provide additional data on the serum levels of IL-6 sR and TGF-β1 in normal healthy ageing.

The effect of age and sex on DHEA-S levels in adults has previously been described [24–27]. This study confirms the results of these earlier findings and clearly demonstrates that in normal healthy male and female blood donors up to the age of 70 years, serum DHEA-S levels significantly decline with age. Although we did not carry out a longitudinal study, it is worth noting that within an individual the levels of DHEA-S may fluctuate over time but the general trend is a decline [43]. Despite the high variability of serum DHEA-S levels among individuals, we were able to demonstrate a highly significant difference between the sexes ($P = 0{\cdot}0001$). Previous studies suggest that this variation is largely due to differences in the DHEA-S clearance rate between men and women [44]. Our results demonstrate that the DHEA-S levels peaked at age 20–29 years in males and at 17–19 years in females, which is in agreement with earlier studies [24]. We also noted a slight rise in the mean DHEA-S levels in women at age 35–44 years. This result was apparent where we shifted the decades slightly and re-examined the data in the 17–24 (5·17 μM), 25–34 (4·37 μM), 35–44 (4·61 μM), 45–54 (3·10 μM), 55–64 (2·04 μM) and 65–74 year (1·83 μM) age groups. A recent study has suggested that this is due to a premenopausal rise in DHEA [27].

Our study of the circulating levels of IL-6 demonstrate the problems associated with measuring this molecule in normal healthy donors using standard clinical assays. Approximately 60% of all samples we assayed had IL-6 levels which fell below the reporting range for the Immulite assay. Previously we demonstrated that a relatively high number of normal healthy samples had undetectable levels of IL-6 using a standard ELISA [21]. In the present study we found no evidence to suggest an age-related increase in circulating IL-6 using the Immulite assay. It was only when we carried out a high sensitivity IL-6 assay that we could demonstrate an age-related increase in IL-6. Furthermore, this relationship was found to be significant only in males. This result agrees with a previous report on a small group of samples, which found that plasma IL-6 was higher in older subjects compared with younger ones, the relationship being only significant in males [9]. However, our results contrast with a recent study using a similar high sensitivity assay and similar numbers of samples, which suggested that serum IL-6 was significantly positively correlated with age in both males and females [23]. It is worth noting, however, that the aforementioned study included samples from individuals between 70 and 80 years of age, and therefore a direct comparison is not possible. It may be that such differences become apparent in much older age groups, because we too have observed a higher incidence of IL-6 in a small number of samples from adults over 70 years of age (results not shown).

Although all of the sera analysed in the present study were obtained from individuals who met the strict criteria laid down by the Blood Transfusion Service in the UK, we can not rule out the possibility that the occasional high IL-6 value observed was due to some undetectable subclinical infection or disease not apparent at the time of sampling. This is obviously a matter which needs to be taken into consideration when interpreting this type of data, since earlier reports have suggested that plasma and serum IL-6 levels are relatively sensitive to health status [8,45]. However, in our present study we did find a higher incidence of these higher IL-6 levels in males who were in the older age groups.

Several other groups have measured plasma IL-6 in healthy young and old subjects, combining their data for males and females, and found significantly higher IL-6 levels in older subjects [11,13]. Others have suggested that serum IL-6 levels are similar in healthy young and elderly individuals. For example, one group found the circulating levels of IL-6 in a group of 26 elderly individuals with a mean age of 79 years to be indistinguishable from the levels found in a group of 13 young individuals with a mean age of 29·9 years [35]. It is unclear why these discrepancies in the literature exist, but they can partly be explained by differences in assay methods, sample sizes, health and ages of volunteers, and combining the results obtained from males and females. For example, one group used a bioassay to measure IL-6 and assayed samples from only 20 young and 18 elderly volunteers aged 21–99 years, and also combined the results from males and females [11]. Another group measured IL-6 using a commercial ELISA in samples from people in the range 23–87 years old, again combining their data from males and females [13]. It is, however, worth noting that previous studies generally agree with our finding that circulating IL-6 levels do not vary significantly between the sexes [23,35].

We found that serum IL-6 levels were significantly inversely correlated with serum DHEA-S levels in healthy male subjects but not in female subjects. This result is in contrast with a recent report which found significant negative correlations between serum IL-6 and DHEA-S in both males and females [23]. We also observed a similar association in a small number of paediatric and geriatric samples (results not shown). We found that children under 7 years old and adults over 70 years old had an extremely low DHEA-S serum level but a relatively high IL-6 serum level (both males and females). In general, it would appear that samples with high DHEA-S serum levels have a corresponding low IL-6 serum levels and *vice versa*. In this regard, our results may lend support to those claims which suggest the usefulness of DHEA replacement therapy in the treatment of age-related immune dysfunctions [20,29–34].

As far as we are aware there has been no previous attempt to measure IL-6 sR and only one attempt to measure TGF-β1 [35] levels throughout adulthood in normal healthy individuals. We failed to demonstrate any age- or gender-related differences in either of these molecules in either male or female blood donors between 20 and 59 years of age. Of interest is the fact that circulating IL-6 sR levels were found to be approximately 10 000 times higher than the corresponding circulating IL-6 levels. This might suggest the rapid neutralization of IL-6 in the circulation by its soluble receptor, but as yet there is no experimental proof to support this. Of note was our observation that there was a significant negative correlation between circulating DHEA-S and IL-6 sR in males. However, we are at a loss to explain the significance of this finding and clearly it warrants further investigation. Our TGF-β1 results agree with and extend a previous study which reported that the levels of this molecule were similar in serum samples from healthy young individuals (mean age 30 years) and healthy elderly individuals (mean age 79 years). However, it is worth noting that they used a TGF-β1 bioassay and appeared to find much lower levels than we found using an ELISA. While it has previously been suggested that DHEA can induce TGF-β1 production in mitogen-stimulated murine macrophages [37], our results indicate that the circulating levels of this anti-inflammatory cytokine do not vary with age within the age range 20–59 years, unlike DHEA-S levels which decline by approximately 50%.

Overall, our findings, at least in males, are consistent with the idea that ageing is associated with a significant decrease in the circulating levels of DHEA-S and a slight increase in the circulating levels of IL-6. Furthermore, we can speculate on the possibility of gender-related differences in the control of IL-6 levels by DHEA between males and females. In comparison, we found that the circulating levels of IL-6 sR and TGF-β1 did not significantly change with ageing in either males or females. Nevertheless, our findings in males lend support to the suggestion that the production of IL-6 is influenced by DHEA-S [20,22,23]. However, our results clearly highlight the complex nature of the relationship between these molecules in the ageing process, and demonstrate the need to use high sensitivity assays when measuring IL-6 in apparently healthy individuals under the age of 70 years.

In conclusion, the relationship between DHEA-S, IL-6 and IL-6 sR appears to be much more complex than previously appreciated and further studies are clearly warranted. In this regard we believe that particular attention should be devoted to investigating the influence of DHEA on the production of IL-6 in isolated lymphocytes from young and old individuals *in vitro*. Although previous reports have studied the effects of DHEA on IL-6 secretion *in vitro* [21,23], we are currently undertaking studies on the effects of DHEA on the transcriptional control of IL-6 expression and hope to report our findings in the near future.

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REFERENCES

1 Pawelec G, Adibzadeh M, Pohla H, Schaudt K. Immunosenescence: ageing of the immune system. Immunol Today 1995; **16**:420–2.

2 Burns EA, Goodwin JS. Immunodeficiency of aging. Drugs Aging 1997; **11**:374–97.

3 Candore G, Di Lorenzo G, Melluso M, Cigna D, Colucci AT, Modica MA, Caruso C. γ-Interferon, interleukin-4 and interleukin-6 in vitro production in old subjects. Autoimmunity 1993; **16**:275–80.

4 Cakman I, Rohwer J, Schütz RM, Kirchner H, Rink L. Dysregulation between TH_1 and TH_2 T cell subpopulations in the elderly. Mech Ageing Dev 1996; **87**:197–209.

5 Caruso C, Candore G, Cigna D, Di Lorenzo G, Sireci G, Dieli F, Salerno A. Cytokine production pathway in the elderly. Immunol Res 1996; **15**:84–90.

6 Spencer NFL, Norton SD, Harrison LL, Li GZ, Daynes RA. Dysregulation of IL-10 production with aging: possible linkage to the age-associated decline in DHEA and its sulfated derivative. Exp Gerontol 1996; **31**:393–408.

7 Gillis S, Kozak R, Durante M, Weksler ME. Immunological studies of

aging: decreased production of and response to T cell growth factor by lymphocytes from aged humans. J Clin Invest 1981; **67**:937–42.
8 Mysliwska J, Bryl E, Foerster J, Mysliwski A. Increase of interleukin 6 and decrease of interleukin 2 production during the ageing process are influenced by health status. Mech Ageing Dev 1998; **100**:313–28.
9 Wei J, Xu H, Davies JL, Hemmings GP. Increase of plasma IL-6 concentration with age in healthy subjects. Life Sci 1992; **51**:1953–6.
10 Ershler WB. Interleukin-6: a cytokine for gerentologists. J Am Geriatr Soc 1993; **41**:176–81.
11 Ershler WB, Sun WH, Binkley N *et al*. Interleukin-6 and aging: blood levels and mononuclear cell production increase with advancing age and *in vitro* production is modifiable by dietary restriction. Lymphokine Cytokine Res 1993; **12**:225–30.
12 Fagiolo U, Cossarizza A, Scala E *et al*. Increased cytokine production in mononuclear cells of healthy elderly people. Eur J Immunol 1993; **23**:2375–8.
13 Hager K, Machein U, Krieger S, Platt D, Seefried G, Bauer J. Interleukin-6 and selected plasma proteins in healthy persons of different ages. Neurobiol Aging 1994; **15**:771–2.
14 Dasgupta B, Corkill M, Kirkham B, Gibson T, Panayi G. Serial estimation of interleukin 6 as a measure of systemic disease in rheumatoid arthritis. J Rheumatol 1992; **19**:22–25.
15 Manolagas SC, Jilka RL. Bone marrow, cytokines, and bone remodeling. N Engl J Med 1995; **332**:305–11.
16 Ikeda U, Ikeda M, Seino Y, Takahashi M, Kano S, Shimada K. Interleukin 6 gene transcripts are expressed in atherosclerotic lesions of genetically hyperlipidemic rabbits. Atherosclerosis 1992; **92**:213–8.
17 Vandenabeele P, Fiers W. Is amyloidogenesis during Alzheimer's disease due to an IL-1-/IL-6-mediated 'acute phase response' in the brain? Immunol Today 1991; **12**:217–9.
18 Wood JA, Wood PL, Ryan R, Graff-Radford NR, Pilapil C, Robitaille Y, Quirion R. Cytokine indices in Alzheimer's temporal cortex: no changes in mature IL-1β or IL-1RA but increases in the associated acute phase proteins IL-6, α2-macroglobulin and C-reactive protein. Brain Res 1993; **629**:245–52.
19 Kawano M, Hirano T, Matsuda T *et al*. Autocrine generation and requirement of BSF-2/IL-6 for human multiple myelomas. Nature 1988; **332**:83–85.
20 Daynes RA, Araneo BA, Ershler WB, Maloney C, Li GZ, Ryu SY. Altered regulation of IL-6 production with normal aging. J Immunol 1993; **150**:5219–30.
21 James K, Premchand N, Skibinska A, Skibinski G, Nicol M, Mason JI. IL-6, DHEA and the ageing process. Mech Ageing Dev 1997; **93**:15–24.
22 Reed MJ. The discriminant-function test: a marker of Th1/Th2 cell cytokine secretion and breast tumour oestrogen synthesis. Mol Med Today 1995; **1**:98–103.
23 Straub RH, Konecna L, Hrach S, Rothe G, Kreutz M, Schölmerich J, Falk W, Lang B. Serum dehydroepiandrosterone (DHEA) and DHEA sulfate are negatively correlated with serum interleukin-6 (IL-6), and DHEA inhibits IL-6 secretion from mononuclear cells in man *in vitro*: possible link between endocrinosenescence and immunosenescence. J Clin Endocrinol Metab 1998; **83**:2012–7.
24 Orentreich N, Brind JL, Rizer RL, Vogelman JH. Age changes and sex differences in serum dehydroepiandrosterone sulfate concentrations throughout adulthood. J Clin Endocrinol Metab 1984; **59**:551–5.
25 Bélanger A, Candas B, Dupont A, Cusan L, Diamond P, Gomez JL, Labrie F. Changes in serum concentrations of conjugated and unconjugated steroids in 40- to 80-year-old men. J Clin Endocrinol Metab 1994; **79**:1086–90.
26 Labrie F, Bélanger A, Cusan L, Gomez JL, Candas B. Marked decline in serum concentrations of adrenal C19 sex steroid precursors and conjugated androgen metabolites during aging. J Clin Endocrinol Metab 1997; **82**:2396–402.
27 Sulcová J, Hill M, Hampl R, Stárka L. Age and sex related differences in serum levels of unconjugated dehydroepiandrosterone and its sulphate in normal subjects. J Endocrinol 1997; **154**:57–62.
28 Romanoff LP, Morris CW, Welch P, Rodriguez RM, Pincus G. The metabolism of cortisol-4-C^{14} in young and elderly men. 1. Secretion rate of cortisol and daily excretion of tetrahydrocortisol, allotetrahydrocortisol, tetrahydrocortisone and cortolone (20α and 20β). J Clin Endocrinol Metab 1961; **21**:1413–25.
29 Daynes RA, Araneo BA. Prevention and reversal of some age-assocated changes in immunologic responses by supplemental dehydroepiandrosterone sulfate therapy. Aging Immunol Infect Dis 1992; **3**:135–54.
30 Casson PR, Andersen RN, Herrod HG, Stentz FB, Straughn AB, Abraham GE, Buster JE. Oral dehydroepiandrosterone in physiologic doses modulates immune function in postmenopausal women. Am J Obstet Gynecol 1993; **169**:1536–9.
31 Yen SSC, Morales AJ, Khorram O. Replacement of DHEA in aging men and women: potential remedial effects. Ann NY Acad Sci 1995; **774**:128–42.
32 Khorram O. DHEA: a hormone with multiple effects. Curr Opin Obs Gynecol 1996; **8**:351–4.
33 Watson RR, Huls A, Araghinikuam M, Chung S. Dehydroepiandrosterone and diseases of aging. Drugs Aging 1996; **9**:274–91.
34 Khorram O, Vu L, Yen SC. Activation of immune function by dehydroepiandrosterone (DHEA) in age-advanced men. J Gerontol 1997; **52A**:M1–M7.
35 Peterson PK, Chao CC, Carson P, Hu S, Nichol K, Janoff EN. Levels of tumor necrosis factor α, interleukin 6, interleukin 10, and transforming growth factor β are normal in the serum of the healthy elderly. Clin Infect Dis 1994; **19**:1158–9.
36 Müller-Newen G, Küster A, Hemmann U *et al*. Soluble IL-6 receptor potentiates the antagonistic activity of soluble gp130 on IL-6 responses. J Immunol 1998; **161**:6347–55.
37 Wu MF, Chang HL, Tseng J. Dehydroepiandrosterone induces the transforming growth factor-β production by murine macrophages. Int J Tiss Reac 1997:141–8.
38 Wagstaff W. Guidelines for the Blood Transfusion Services in the United Kingdom, 3rd edn. Watford: National Blood Service, 1996.
39 Babson AL. The Cirrus Immulite automated immunoassay system. J Clin Immunoassay 1991; **14**:83–88.
40 Babson AL, Olson DR, Palmieri T, Ross AF, Becker DM, Mulqueen PJ. The Immulite assay tube: a new approach to heterogeneous ligand assay. Clin Chem 1991; **37**:1521–2.
41 Rosenfeld RS, Hellman L, Gallagher TF. Metabolism and interconversion of dehydroisoandrosterone and dehydroisoandrosterone sulfate. J Clin Endocrinol Metab 1972; **35**:187–93.
42 Longcope C. Dehydroepiandrosterone metabolism. J Endocrinol 1996; **150**:S125–S127.
43 Orentreich N, Brind JL, Vogelman JH, Andres R, Baldwin H. Long-term longitudinal measurements of plasma dehydroepiandrosterone sulfate in normal men. J Clin Endocrinol Metab 1992; **75**:1002–4.
44 Zumoff B, Bradlow HL. Sex differences in the metabolism of dehydroisoandrosterone sulfate. J Clin Endocrinol Metab 1980; **51**:334–6.
45 Cohen HJ, Pieper CF, Harris T, Rao KMK, Currie MS. The association of plasma IL-6 levels with functional disability in community-dwelling elderly. J Gerontol: Med Sci 1997; **52A**:M201–M208.